Certificate of Analysis

Certified Reference Material

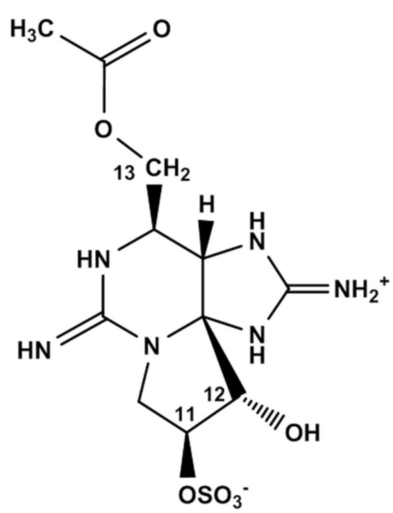
CRM-LWTX1 (Lot# 20101027)

Certified Calibration Solution for *Lyngbya wollei* Toxin 1

Lyngbya wollei Toxin 1 (LWTX1) is an analogue of saxitoxin found in the filamentous cyanobacterium *Lyngbya wollei* [1]. CRM-LWTX1 is a certified instrument calibration solution prepared to aid the analyst in the determination of LWTX1. Each ampoule contains a solution of LWTX1 dissolved in filtered, aqueous acetic acid (17 µM, pH 5).

Table 1: Certified concentration and uncertainty for CRM-LWTX1.

Compound	µmol/L (at +24°C)
LWTX1	11.0 ± 0.7



13-*O*-acetyl-12β-deoxydecarbamoylsaxitoxin-11β-*O*-sulfate (LWTX1)
CAS registry no.: 200816-95-1
Molecular formula: $C_{11}H_{18}N_6O_7S$
Molecular weight: 378.4 g/mol
$[M+H]^+$: *m/z* 379.1030

Period of validity: 1 year from date of sale
Storage conditions: -12 °C or below

Intended Use

CRM-LWTX1 is a calibration solution CRM designed for analytical method development and accurate quantitation of LWTX1. The concentration is suitable for preparing a dilution series for calibration of instruments such as liquid chromatography with detection by post-column oxidation-fluorescence (LC-ox-FLD) or liquid chromatography-mass spectrometry (LC-MS).

Instructions for Storage and Use

To ensure the stability of CRM-LWTX1, ampoules should be stored at -12 °C or below.

Prior to opening, each ampoule should be allowed to warm to room temperature and the contents thoroughly mixed. The ampoule should be opened at the pre-scored mark. Once an ampoule has been opened, accurate aliquots should be removed with calibrated volumetric equipment and transferred to volumetric flasks or vials. An increase in concentration due to evaporation of solvent will occur if the solution is left opened for more than a few minutes. It is recommended that solutions of CRM-LWTX1 should not be evaporated to dryness due to potential losses. *Note:* The volume of the solution is not certified; only the concentration is certified. Therefore, the entire contents of the ampoule should not simply be transferred to a volumetric flask and diluted to volume.

Preparation of CRM-LWTX1

A sample of *Lyngbya wollei* was collected from the St. Lawrence River (Canada) and provided by Environment Canada [2]. LWTX1 was isolated from this material using preparative scale chromatography. Purified LWTX1 was dried *in vacuo* and dissolved in filtered, aqueous acetic acid (17 µM, pH 5) to give a stock solution. The purity of the toxin was checked by 500 MHz proton nuclear magnetic resonance (NMR) spectroscopy (Figure 1), LC-ox-FLD (Figure 2) [3], LC-MS(MS) (Figures 3 and 4) [4], capillary electrophoresis with UV detection (CE-UVD) [5], and liquid chromatography with chemiluminescence nitrogen detection (LC-CLND) [6].

The CRM-LWTX1 solution was prepared by making an accurate dilution of the quantitative NMR stock solution in filtered (0.2 µm) and degassed acetic acid (17 µM, pH 5). Aliquots were dispensed into clean argon-filled amber glass ampoules and immediately flame-sealed. Each ampoule contains approximately 0.5 mL of solution.

Analytical Methods and Value Assignment

The certified value for CRM-LWTX1 (Table 1) is based on results obtained at NRC using two analytical methods: (a) quantitative ^{1}H-NMR (qNMR) [7], and (b) LC-CLND [6], using accurate caffeine solutions as the calibrant.

CRM-LWTX1 contains small amounts of a deacylated analog, 12β-deoxydecarbamoylsaxitoxin-11β-*O*-sulfate, and an analog missing an oxygen at C12, 13-*O*-acetyl-12-dideoxydecarbamoylsaxitoxin-11β-*O*-sulfate. Their concentrations were estimated using LC-MS with CRM-LWTX1 as the calibrant and assuming equimolar responses (Table 2).

Table 2: Information values for other compounds present in CRM-LWTX1 at the time of packaging.

Analogue	Peak No.	$[M+H]^+$	µmol/L*
12β-deoxydecarbamoylsaxitoxin-11β-*O*-sulfate	1	337.1	0.77
13-*O*-acetyl-12-dideoxydecarbamoylsaxitoxin-11β-*O*-sulfate	2	363.1	0.37

* These concentrations are not certified.

Homogeneity

A representative number of CRM-LWTX1 ampoules (n = 14) were selected from across the fill series and LWTX1 concentrations measured using LC-ox-FLD. No heterogeneity was observed.

Stability

Stability studies demonstrated good long-term stability of CRM-LWTX1 with no detectable decomposition observed over a period of 18 months at -25 °C.

Uncertainty

All reasonable sources of error related to the characterization of CRM-LWTX1 were considered and measured. The overall uncertainty estimate (U_{CRM}) includes uncertainties associated with batch characterization (u_{char}), between-bottle variation (u_{hom}) and instability during storage (u_{stab}) [8-11]. A combined uncertainty component relating to the two analytical methods used is included in u_{char}. The between-bottle variation (u_{hom}) was found to be negligible. These components are listed in Table 3, and are combined and expanded as follows:

$$U_{CRM} = k\sqrt{u_{char}^2 + u_{hom}^2 + u_{stab}^2}$$

where k is the coverage factor for a 95% confidence level (= 2).

Table 3: Uncertainty components for the certified value of CRM-LWTX1.

Uncertainties	[µmol/L]
u_{char}	0.31
u_{hom}	*NA*
u_{stab}	0.06

Safety Instructions

LWTX1 has been found to have relatively low toxicity [1]. Nevertheless, only qualified personnel should handle the solution and appropriate disposal methods should be used. Heavy gloves and eye protection should be used when opening the ampoule in the event the glass shatters. A safety data sheet (SDS) is available for CRM-LWTX1.

Period of Validity

If stored unopened at the recommended storage condition of -12 °C or below, the certified concentration of CRM-LWTX1 is valid for 1 year from the date of sale.

Metrological Traceability

Results presented in this certificate are traceable to the SI (*Système international d'unités*) through gravimetrically prepared standards of established purity.

Quality System (ISO/IEC 17025, ISO Guide 34)

This material was produced in compliance with the documented National Research Council of Canada (NRC) Measurement Science and Standards (MSS) Quality System, which conforms with the requirements of ISO/IEC 17025 and ISO Guide 34.

The MSS Quality System supporting NRC calibration and measurement capabilities, as listed in the Bureau international des poids et mesures (BIPM) key comparison database (http://kcdb.bipm.org/), has been reviewed and approved under the authority of the Inter-American Metrology System (SIM) and found to be in compliance with the expectations of the Comité international des poids et mesures (CIPM) Mutual Recognition Arrangement. The SIM certificate of approval is available upon request.

References

1. Onodera H, Satake M, Oshima Y, Yasumoto T, Carmichael WW (1997). New saxitoxin analogues from the freshwater filamentous cyanobacterium *Lyngbya wollei*. *Nat Toxins* 5: 146-151.
2. Lajeunesse A, Segura PA, Gélinas M, Hudon C, Thomas K, Quilliam MA, Gagnon C (2012). Detection and confirmation of saxitoxin analogues in freshwater benthic *Lyngbya wollei* algae collected in the St. Lawrence River (Canada) by liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 1219: 93-103.
3. van de Riet J, Gibbs RS, Muggah PM, Rourke WA, MacNeil JD, Quilliam MA (2011). Liquid chromatography post-column oxidation (PCOX) method for the determination of paralytic shellfish toxins in mussels, clams, oysters, and scallops: collaborative study. *J AOAC Int* 94: 1154-1176.
4. Thomas KM, Beach DG, Reeves KL, Gibbs RS, Kerrin ES, McCarron P, Quilliam MA (2017). Hydrophilic interaction liquid chromatography-tandem mass spectrometry for quantitation of paralytic shellfish toxins: validation and application to reference materials. *Anal Bioanal Chem* 409: 5675-5687.
5. Thibault P, Pleasance S, Laycock MV (1991). Analysis of paralytic shellfish poisons by capillary electrophoresis. *J Chromatogr A* 542: 483-501.
6. Burton IW, Quilliam MA, Walter JA (2005). Quantitative ^{1}H NMR with external standards: use in preparation of calibration solutions for algal toxins and other natural products. *Anal Chem* 77: 3123-3131.
7. Thomas K, Wechsler D, Chen YM, Crain S, Quilliam MA (2016). Analysis of natural toxins by liquid chromatography-chemiluminescence nitrogen detection and application to the preparation of certified reference materials. *J AOAC Int* 99: 1173-1184.
8. Pauwels J, Lamberty A, Schimmel H (2000). Evaluation of uncertainty of reference materials. *Accred Qual Assur* 5: 95-99.
9. Pauwels J, Lamberty A, Schimmel H (1998). The determination of the uncertainty of reference materials certified by laboratory intercomparison. *Accred Qual Assur* 3: 180-184.
10. Ellison SLR, Burke S, Walker RF, Heydorn K, Mansson M, Pauwels J, Wegscheider W, te Nijenhuis B (2001). Uncertainty for reference materials certified by interlaboratory study: Recommendations of an international study group. *Accred Qual Assur* 6: 274-277.
11. Evaluation of measurement data – Guide to the expression of uncertainty in measurement (GUM), JCGM 100:2008 (corrected version 2010), www.bipm.org. Printed as ISO/IEC Guide 98-3:2008, ISO Geneva.

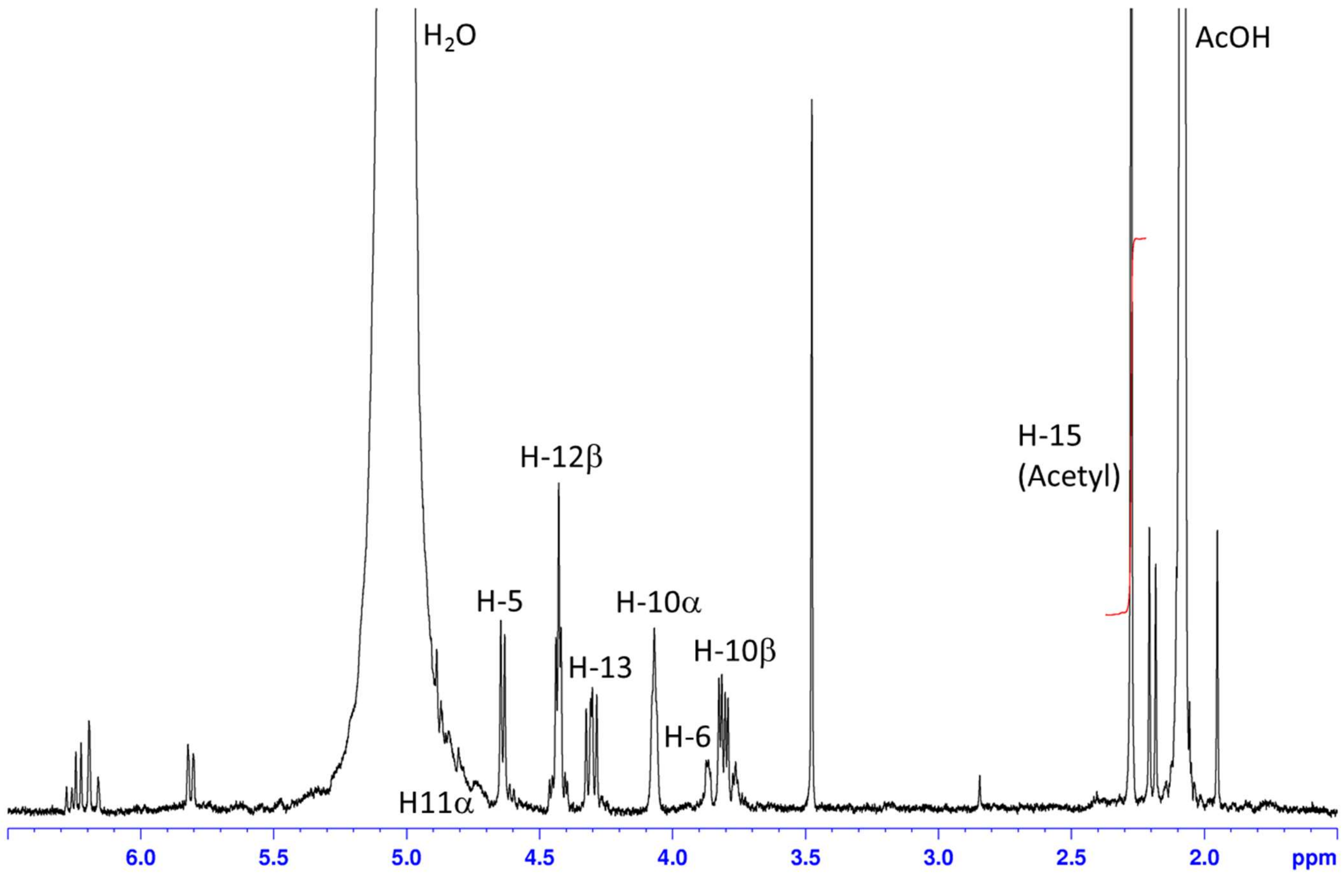


Figure 1: Proton NMR spectrum of the stock solution of LWTX1 in aqueous acetic acid (17 µM, pH 5).

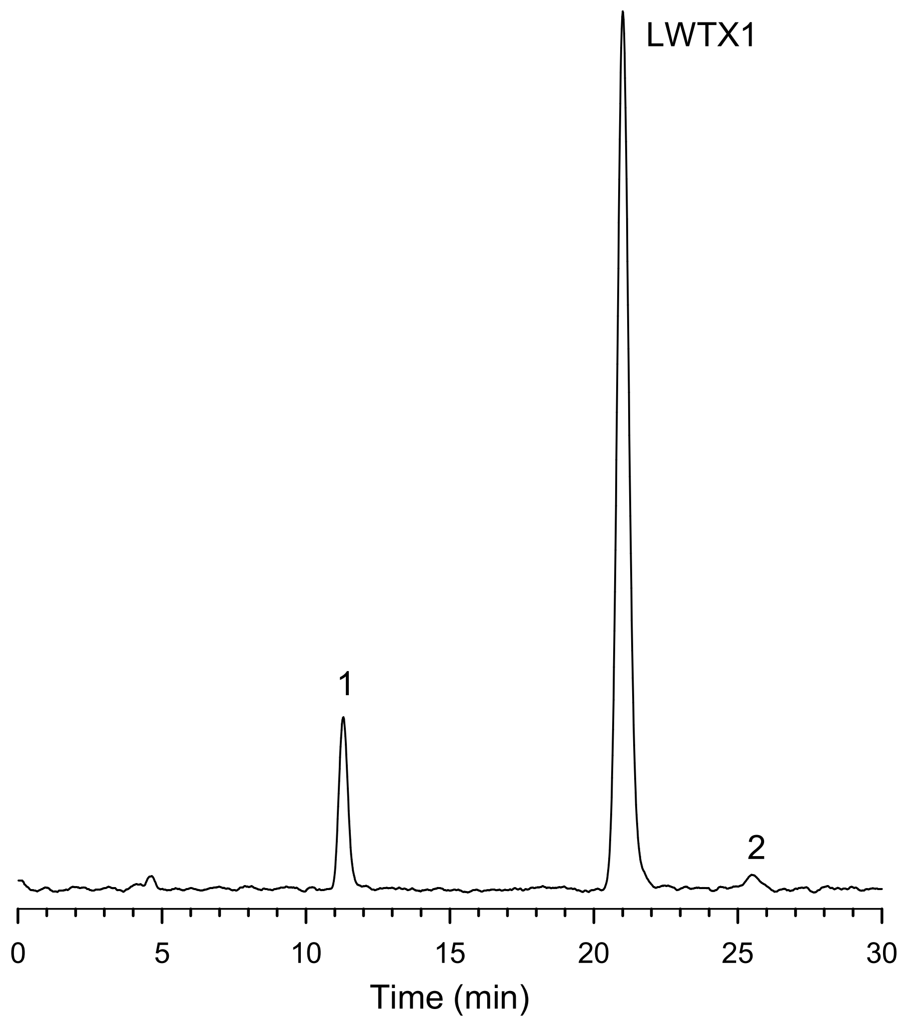


Figure 2: LC-ox-FLD analysis of CRM-LWTX1. Identities of 1 and 2 are given in Table 2. Conditions: Zorbax Bonus-RP column (150 × 4.6 mm i.d., 5 µm) at +35 °C; 0.8 mL/min of 5.5 mM ammonium phosphate with 11 mM sodium heptane sulphonate, pH 7.1; 25 µL injection; post column oxidation using 0.4 mL/min 5 mM periodic acid in 100 mM sodium phosphate at pH 7.8; reaction coil at +80 °C; effluent acidified with 0.4 mL/min 0.75 M nitric acid; fluorescence detection with excitation at 330 nm and emission at 390 nm.

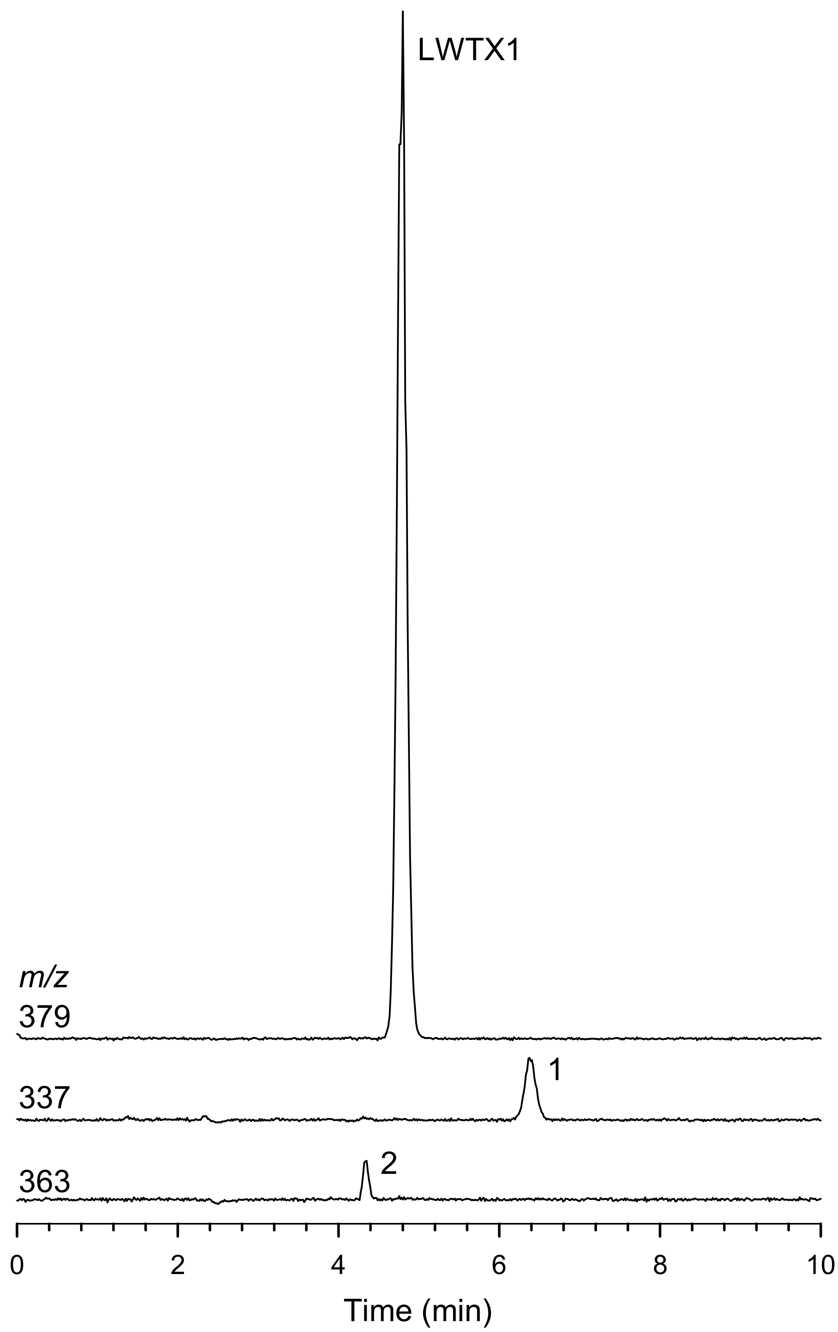


Figure 3: LC-MS analysis of CRM-LWTX1 using selected ion monitoring (SIM). Identities of 1 and 2 are given in Table 2. Conditions: Agilent 1260 LC and Sciex API5500 QTRAP MS; 30 V declustering potential; 0.5 µL injection volume; 5 µm TOSOH Amide-80 column (250 × 2 mm i.d.) at +40 °C; isocratic elution at 0.2 mL/min flow with 70% B; mobile phase A: water with 50 mM HCOOH and 2 mM NH_4COOH; mobile phase B: CH_3CN.

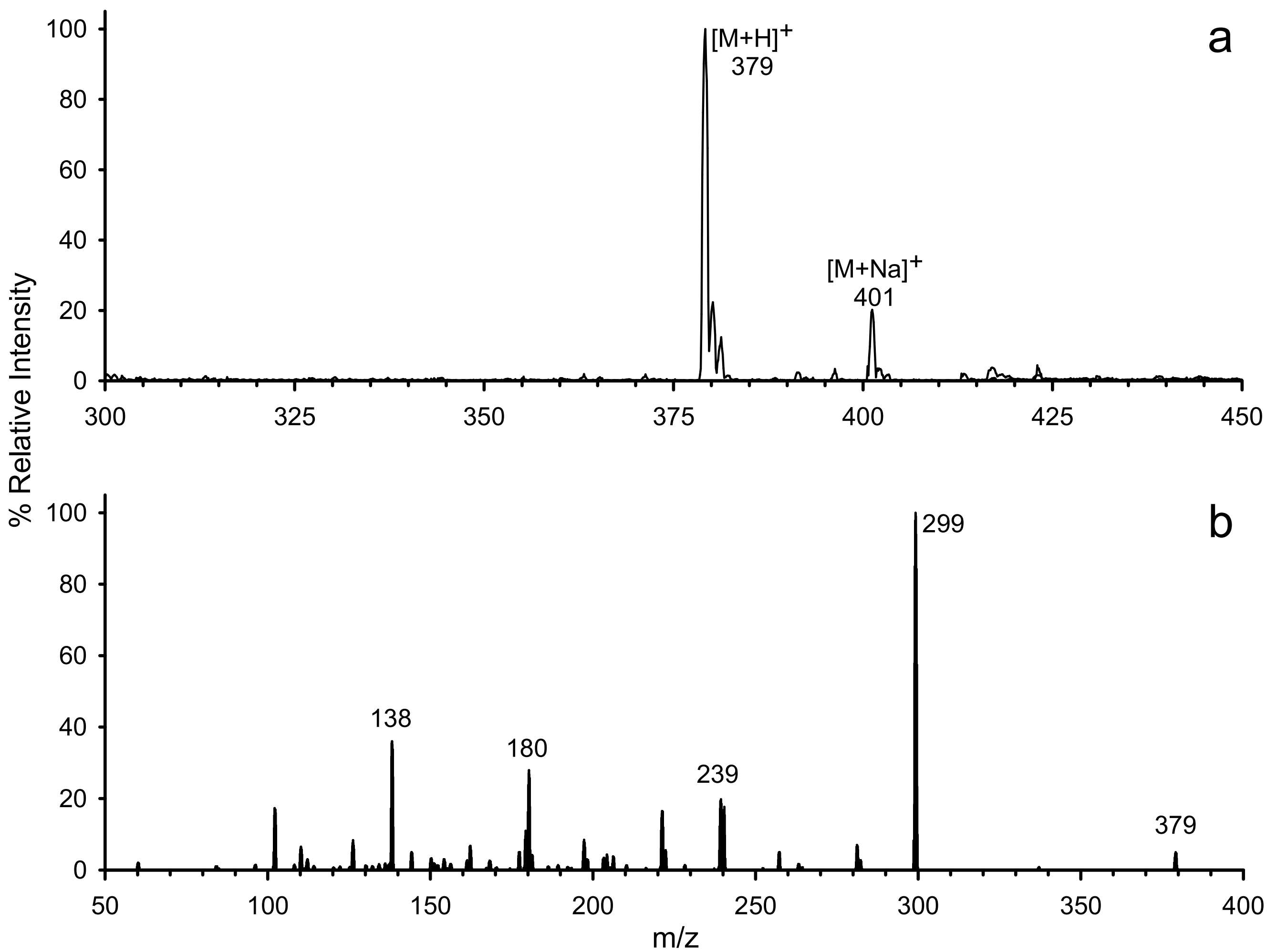


Figure 4: Mass spectra of LWTX1 generated from analysis of CRM-LWTX1: full Q1 scan positive ion ESI mass spectrum (a); product ion spectrum of *m/z* 379 with collision energy (CE) = 35 V (b). Conditions: Agilent 1200 LC and Sciex API4000 QTRAP MS; 70 V declustering potential; 2.5 µL injection volume; 5 µm TOSOH Amide-80 column (250 × 2 mm i.d.) at +40 °C; flow = 0.2 mL/min; mobile phase A: water with 50 mM HCOOH and 2 mM NH_4COOH; mobile phase B: CH_3CN; gradient elution 10 to 45% A at 25 min, to 70% A at 27 min, hold to 40 min.

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